



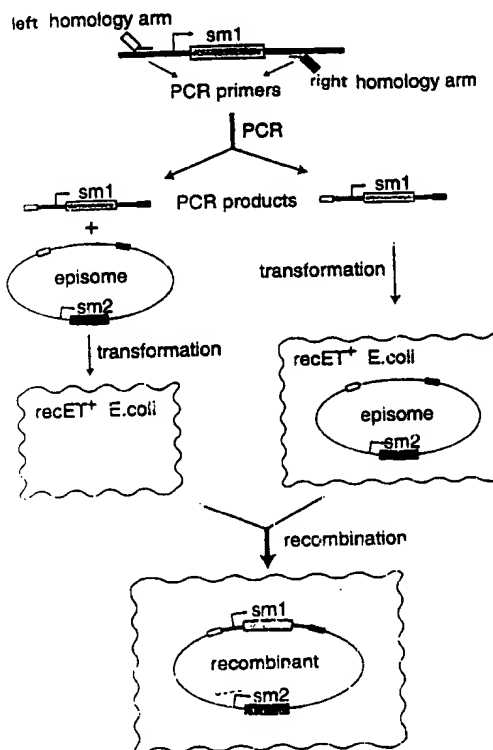
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(54) Title: NOVEL DNA CLONING METHOD RELYING ON THE E. COLI REC^E/REC^T RECOMBINATION SYSTEM

(57) Abstract

The invention refers to a novel method for cloning DNA molecules using a homologous recombination mechanism between at least two DNA molecules comprising: a) providing a host cell capable of performing homologous recombination, b) contacting in said host cell a first DNA molecule which is capable of being replicated in said host cell with a second DNA molecule comprising at least two regions of sequence homology to regions on the first DNA molecule, under conditions which favour homologous recombination between said first and second DNA molecules and c) selecting a host cell in which homologous recombination between said first and second DNA molecules has occurred. In particular, it relies on the use of the E. coli RecE and RecT proteins, the bacteriophage Red-alpha and Red-beta proteins, or the phage P22 recombination system. The beneficial effects of concomitant expression of the RecBC inhibitor genes (e.g. Red-Gamma) is also exemplified.



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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

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IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	OLINER J.D. ET AL.: "In vivo Cloning of PCR Products in E. coli" NUCLEIC ACIDS RESEARCH, vol. 21, no. 22, 1993, pages 5192-5197, XP002064297 cited in the application	1-6, 8-13, 19, 20, 22-27, 34, 35, 37-42, 46-48, 50 18, 28
Y	See page 5192, column 2, line 8 - line 31, and discussion section	
Y	NUSSBAUM A ET AL: "Restriction-stimulated homologous recombination of plasmids by the RecE pathway of Escherichia coli" GENETICS, vol. 130, no. 1, January 1992, pages 37-49, XP002103370 see figures 1,7	18, 28

☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 98/07945

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	ZHANG Y ET AL: "A new logic for DNA engineering using recombination in Escherichia coli" NATURE GENETICS, vol. 20, no. 2, October 1998, pages 123-128, XP002103371 see the whole document ---	1-50
A	KOLODNER R ET AL: "Homologous pairing proteins encoded by the Escherichia coli recE and recT genes" MOLECULAR MICROBIOLOGY, vol. 11, no. 1, 1994, pages 23-30, XP002064301 cited in the application ---	
A	LUISI-DELUCA C ET AL: "Genetic and physical analysis of plasmid recombination in recB recC sbcB and recB recC sbcA Escherichia coli K-12 mutants" GENETICS, vol. 122, 1989, pages 269-278, XP002064305 ---	
A	DEGRYSE E: "Evaluation of Escherichia coli recBC sbcBC mutants for cloning by recombination in vivo" JOURNAL OF BIOTECHNOLOGY, vol. 2, no. 39, 15 April 1995, page 181-187 XP004036984 ---	
A	YANG X ET AL: "Homologous recombination based modification in Escherichia coli and germ line transmission in transgenic mice of a bacterial artificial chromosome" NATURE BIOTECHNOLOGY, vol. 15, September 1997, pages 859-865, XP002103372 ---	
A	MURPHY K: "Lambda Gam protein inhibits the helicase and Chi-stimulated recombination activities of Escherichia coli RecBCD enzyme" JOURNAL OF BACTERIOLOGY, vol. 173, no. 18, September 1991, pages 5808-5821, XP002103373 -----	